Coliscan Easygel Test Procedure for Detection of Coliforms and E. coli
For accurate test results please read the procedure before beginning the test.

1. Either collect your water sample in a sterile container and transport the water back to your school, or take a measured water sample directly from the source and place directly into the bottle of Coliscan Easygel. Water samples kept longer than one (1) hour prior to plating, or any Coliscan Easygel bottle that has had a sample placed into it for transport longer than ten (10) minutes, should be kept on ice or in a refrigerator until plated.

2. Label the petri dishes with the appropriate sample information. A permanent marker or wax pencil will work.

<table>
<thead>
<tr>
<th>Inoculation of Coliscan Easygel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Sources</strong></td>
</tr>
<tr>
<td>Environmental: river, lake, pond, stream, ditch</td>
</tr>
<tr>
<td>Drinking water: well, municipal, bottled</td>
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</tbody>
</table>

3. Sterilely transfer water using a pipette or eyedropper from the sample containers into the bottles of Coliscan Easygel (Consult the table above for rough guidelines for inoculum amount). Swirl the bottles to distribute the inoculum and then pour the medium/inoculum mixtures into the correctly labeled petri dishes. Place the lids back on to the petri dishes. Gently swirl the poured dish until the entire dish is covered with liquid (but be careful not to splash over the side or on the lid).

4. The dishes may be placed right-side-up directly into a level incubator or warm level spot in the room while still liquid. Solidification will occur in approximately 40 minutes.

5. Incubate your sample at 35 °C (95 °F) for 24 hours, or at room temperature for 48 hours. (See comments on incubation on the other side of this sheet)

6. Inspect the dishes. **To obtain a total coliform count on your sample, count all the pink and purple colonies on the Coliscan dish (disregard any light blue, blue-green or white colonies).** The number of pink and purple colonies will give you a total coliform count for your sample.

6a. **If you want to count just E. coli bacteria,** count just the purple colonies on the Coliscan dish (disregard any pink, light blue, blue-green or white colonies). The number of purple colonies will give you the number of E. coli colonies in your sample.

7. **NOTE: To report bacteria in terms of colonies per 100.0 ml of water, first, divide 100 by the number of ml that you used for your sample. Then, multiply the count in your plate from either step 6 or 6a by the result.** For example, a 5.0 ml sample, 100 / 5 = 20.0. So 4 colonies multiplied by 20.0 will equal 80.0 colonies per 100.0 ml of water.

8. Locate the laminated sheet showing the coliform “Q-value chart” or use the ones on this page. Find the “Q-value” of your result. Is the fecal coliform level in the river sample healthy or unhealthy?
**Comments on Incubation:**
Coliscan can effectively differentiate general coliforms from *E. coli* when incubated at either room temperatures or at elevated temperatures (such as 90-98 °F). However, some further explanation may be helpful.

There is no one standard to define room temperature. Most would consider normal room temperature to vary from 68-74 °F, but even within this range the growth of bacteria will be varied. It is a very easy task to make an adequate incubator from a box with a 40-60 watt bulb in it to provide heat at an even rate.

Our general instructions indicate that incubation times for coliforms* (including *E. coli*) are generally 24-48 hours at elevated temperatures (90-98 °F) and 48 or more hours at room temperatures. At elevated temperatures, no counts should be made after 48 hours as any coliforms present will be quite evident by that time and if new colonies form after 48 hours they are most likely not coliforms, but some other type of slow growing organisms that should not be included in your data.

At room temperatures, the best procedure is to watch the plates by checking them at 10-12 hour intervals until you observe some pink or purple colonies starting to form and then allowing another 24-30 hours for the maturation of those colonies. Since the coliforms (including *E. coli*) are generally the faster growing organisms, these will be the first to grow and be counted. Colonies that may show up at a later time are likely to not be coliforms.

As you can see, there are advantages to incubating your dishes at elevated temperatures. First, you can count the results earlier. At 95 °F, it is often possible to do accurate counts at 18-20 hours of incubation. There is also less probability of variation from batch to batch when the incubation temperatures are kept at one uniform level. And a higher incubation temperature will tend to inhibit the growth of non-coliforms that may prefer lower temperatures.

*E. coli* is the primary fecal coliform, however, *Klebsiella* is sometimes of fecal origin. Other general coliform genera include *Enterobacter* and *Citrobacter*.

**General Notes on Differentiating Coliforms and *E. coli***
Generally, water containing *E. coli* (the fecal contamination indicator organism) should not be used for drinking water unless it is sanitized in some manner. Contact your local health department for guidelines regarding *E. coli* and coliforms in recreational waters. Inform them if you suspect that contamination may be occurring from a specific source.

Colonies which have the blue-green color are not exhibited any β-galactosidase activity (which is evidenced by the pink color). Because of this, they are not considered to be either coliforms or *E. coli* and therefore should be ignored when counting your coliform or *E. coli* colonies. Similarly, colonies which are white are exhibiting neither color-causing enzyme, and should also be ignored.

Colonies on the surface of the plate are exposed to the medium on only the underside of the colony. This causes these colonies to appear with much less of the indicator color. *E. coli* colonies may only have a slight purple tinge to them, and it may appear only in the center of the colony with the remainder of the colony being white. Similarly, coliforms on the surface may be light pink or white with a pink center.

**Comments on Disposal:**
Do one of the following prior to disposal in normal trash:

- Place dishes and Coliscan bottles in a pressure cooker and cook at 15 lbs. for 15 minutes. This is the best method.
- Place dishes and Coliscan bottles in an ovenproof bag, seal it, and heat in an oven at 300 °F for 45 minutes.
- Place dishes and Coliscan bottles in a large pan, cover with water and boil for 45 minutes.
- Place 5.0 ml (about 1 teaspoon) of straight bleach onto the surface of the medium of each plate. Allow to sit at least 5 minutes. Place in a watertight bag and discard in trash.